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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Shaun Atchison et al.

Serial No.: 10/000,473 Filed: October 24, 2001

Examiner: OLSEN, Kaj K. Group Art Unit: 1743

For: CASSETTE ASSEMBLY FOR ELECTROPHORESIS GELS

I hereby certify that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" service under 37 CFR § 1.10 on this date: March 28, 2003 and is addressed to Commissioner for Patents, Washington, D.C. 20231. Express Mail Mailing Label No. EJ622912354US

Frank M. Gasparo, Esq. (Reg. No. 44,700) March 28, 2003 Date

Commissioner for Patents
Washington, D.C. 20231

SUBMISSION OF PRIORITY DOCUMENT

SIR:

Applicants hereby submit a certified copy of the priority document: Australian Provisional Application No. PR 1036 filed on October 26, 2000 in the name of Gradipore Limited.

The Commissioner for Patents is hereby authorized to charge payment of all fees associated with this communication to Deposit Account No. 02-0393.

Respectfully submitted,

Date: March 28, 2003

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Enclosure

AUSTRALIA

Patent Office
Canberra

I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR 1036 for a patent by GRADIPORE LIMITED filed on 26 October 2000.

WITNESS my hand this
Sixteenth day of January 2002

J R Yabsley

JONNE YABSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

AUSTRALIA

Patents Act 1990

Gradipore Limited

PROVISIONAL SPECIFICATION

Invention Title:

Improved electrophoresis gel cassette

The invention is described in the following statement:

Technical Field

This invention relates to the field of electrophoresis. In particular, it relates to an improved cassette for use in pre-cast gels and two-dimensional electrophoresis.

Background Art

Two-dimensional electrophoresis is a well known technique which provides high-resolution separations of extremely complex protein mixtures. A first stage separation is carried out in a first-dimension gel which can be, for example a strip of gel on a solid support, or a rod-shaped gel. The second stage involves separation of the components separated in the first-dimension gel in a second-dimension gel. The second-dimension gel is typically one of a variety of slab gels. The gels used in both the first- and second-dimensions can be composed of any porous polymer matrix solvated in a conducting medium. One suitable gel matrix is crosslinked polyacrylamide. The type of electrophoresis performed in the two dimensions is chosen such that the proteins are separated on the basis of a different molecular property in each dimension. For example, in the first-dimension the proteins can be separated on the basis of isoelectric point and in the second-dimension the proteins can be separated on the basis of molecular weight. Conversly, in the first-dimension the proteins can be separated on the basis of molecular weight and in the second-dimension the proteins can be separated on the basis of isoelectric point. Alternatively, in the first-dimension the proteins can be separated on the basis of molecular weight under non-reducing conditions and in the second-dimension the proteins can be separated on the basis of molecular weight under reducing conditions.

The high resolving power of two-dimensional electrophoresis has resulted in its development into a popular analytical tool. Proteomics, the study of protein expression in whole cells, tissues and organisms is just one area of study where two-dimensional electrophoresis has been invaluable. The requirement for this technique is rapidly growing and both first- and second-dimension gels are now commercially available.

Compared with other gel electrophoresis techniques, two-dimensional electrophoresis presents certain difficulties and limitations. A characteristic of pre-cast electrophoresis gels is that they are user-friendly and although this is the case for many gel types currently on the market second-dimension pre-

cast gels still have some difficulties. It is important that use of pre-cast second-dimension gels is simple and convenient for the operator.

Existing second-dimension gels incorporate a large well to accommodate the first-dimension gel after a separation has been carried out in that gel. The second-dimension gels may also define a small well, to accommodate marker proteins for example. The movement of marker proteins through a gel during the second stage separation can be compared with the movement of the sample components from the first-dimension gel in the second-dimension gel to determine a molecular property of interest. After the first-dimension has been run the first dimension gel needs to be placed in contact with the second-dimension gel using an embedding solution. Generally, a polymer solvated in a conducting medium is used, for example agarose, or polyacrylamide. Agarose is formed from a molten solution through physical association whereas polyacrylamide is formed from a monomer solution through chemical bonding. The embedding solution ensures good electrical contact between the first- and second-dimension gels and also prevents the first-dimension gel from moving during the second-dimension electrophoresis run. However, a problem with this procedure is that the embedding solution can spill over into the small well, if a small well is provided, and set or polymerise in it. This spillage causes problems when protein is loaded. Thus, the operator of the equipment has to spend time trying to clean out the small well. Even then the result is not optimal. Often, the bottom of the well is damaged whilst being cleaned. This can cause a distortion in the protein bands. The sample may not be able to be deposited along the bottom of the well because the polymer is blocking the bottom. This causes the protein bands to be diffuse. There are many problems which can arise from the embedding solution setting or polymerising in the small well of the second-dimension gel. Another common problem with pre-cast gels is well collapse during transportation and storage.

One approach by commercial companies to overcome problems discussed above is to supply a range of pre-cast second-dimension gels to researchers with a two-well comb used to define the wells during the manufacturing process remaining in the gel cassette. The comb provides support to the gel fingers during transport and storage. However the comb has to be removed by the operator before use. Once the comb is removed both wells are exposed, although in the first stage of the second-dimension

run the small well is not required. If it is not guarded then the solution used to embed the first-dimension gel will flow into it and cause problems, as described above.

Thus, in general using commercial pre-cast second dimension gels can be labour intensive and subject to experimental difficulty, which is contrary to the aim that commercial pre-cast gels are quick and easy to use.

The present invention seeks to address the problems discussed above and to alleviate at least some of the difficulties and to provide improved pre-cast gels.

Summary of the Invention

According to the present invention, there is provided a cassette incorporating a cast slab gel for use in electrophoresis, the slab gel defining at least two wells in one side or face of the gel, one of the two wells being relatively larger and the other one well being relatively smaller, a narrow wall or finger separating the relatively smaller well from the larger well characterised in that the wall or finger is at least partially defined by the material forming the cassette and typically is also partly defined by the gel material forming the slab and in that a removable plug is located in the well to protect the same.

By providing a partially solid finger it is not necessary to provide a two-well comb to support the finger during transportation and storage. Instead, a small removable comb is inserted into the small well to prevent it from filling up during the step where the first-dimension gel is embedded. After the first-dimension gel has been embedded into position and the embedding solution has set or polymerised, the small comb can be removed and discarded and the small well can be loaded with protein. This invention allows the operator to use the product immediately and with much greater convenience than with existing pre-cast gels.

Typically, the large well is approximately 7 cm in length.

The material of the slab gel may be any medium that can be used to separate proteins, an example is crosslinked polyacrylamide gel.

The cassette can be made from any suitable material. Typical plastic materials include thermoplastics, such as polymethyl methacrylate, styrene acrylonitrile, polycarbonate, polyethylene naphthalate, polyvinyl chloride,

polyvinylidene chloride or polyethylene tetra-phthalate, or a composite of any of these.

Alternatively, the cassette material may be glass or a combination of glass and plastic. The material used may be coated, for example with a barrier layer which can be either hydrophilic or hydrophobic.

The plug can be made from any suitable material. Typical plastic materials include thermoplastics, such as polymethyl methacrylate, styrene acrylonitrile, polycarbonate, polyethylene naphthalate, polyvinyl chloride, polyvinylidene chloride or polyethylene tetra-phthalate, or a composite of any of these.

Alternatively, the plug material may be glass or a combination of glass and plastic. The material used may be coated, for example with a barrier layer which can be either hydrophilic or hydrophobic.

In a related aspect the invention provides a method of making a pre-cast gel including the steps of:-

a. pouring a gel forming material into a cassette defining an internal gel forming space having an internal projection extending into said space, using a comb having a large projection and an adjacent relatively smaller projection spaced from the larger projection by a gap to define a relatively larger well and a relatively smaller well in the gel with the internal projection located in the gap;

b. allowing the gel forming material to polymerise or set;

c. removing the comb;

d. placing a removable plug in the relatively smaller well.

Brief Description of Drawings

The present invention will now be described by way of example only with reference to the accompanying drawings in which:

Figure 1a shows a gel cassette post-manufacture after the slab has been cast, included is the large comb used to define the wells during manufacture;

Figure 1b shows the cassette after the large comb has been removed and the plug is ready to be inserted; and

Figure 1c shows the cassette with a plug protecting the small well and the large well exposed.

Detailed Description of a Preferred Embodiment

Referring to the drawings, Figure 1a shows a gel cassette generally indicated at 10, comprising two plates with substantially planar walls having two sides 12,14 and upper and lower ends 16,18 arranged in a parallel spaced apart array to form a large gel receiving space therebetween. A slab gel 20 is located in the space. The slab gel is formed from a gel forming material such as acrylamide or agarose which is allowed to polymerise or set while a two well comb 22 is inserted into the upper end 16 of the cassette. The comb 22 defines a large projection 24 and a relatively smaller projection 26 which form a relatively larger well 28 and a relatively smaller well 30 in the gel slab. The larger well 28 is typically five to twenty times longer than the small well 30. As can be seen, the two wells are separated by a wall or finger 32 which is partially formed of gel 34 and partially by a plastic stud 33 which is part of the cassette. The plastic stud extends from one plate of the cassette towards the other plate and also provides a locating means for locating the comb as the two projections 24 and 26 locate either side of it during the gel forming stage.

Once the gel has polymerised or set, the two-well comb is removed leaving the large well 28 and the small well 30. A plug 40 is placed in the small well and the cassette is now ready for shipping, storage or use. The partially solid finger is sufficiently self-supporting to obviate the need to ship or store the cassette with the two-well comb 22 included.

In use, a first dimension gel is placed in the large well after a first dimension separation has been carried out in the gel. The first-dimension gel is then connected to the second-dimension gel typically using a polymer solvated in conducting material, such as agarose. Once the first-dimension gel and the second-dimension gel are joined and the embedding solution is set or polymerised, the small plug can be removed. Protein may be placed in the small well 30 for comparison with the movement of the proteins in the first-dimension gel as they travel down through the slab gel under the influence of an electric field.

The cassette, comb and plug will be made of any suitable material. Suitable materials can include plastic, glass, coated materials or combinations of glass, plastic or coated materials.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this twenty-sixth day of October 2000

Gradipore Limited
Patent Attorneys for the Applicant:

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FIGURE 1a

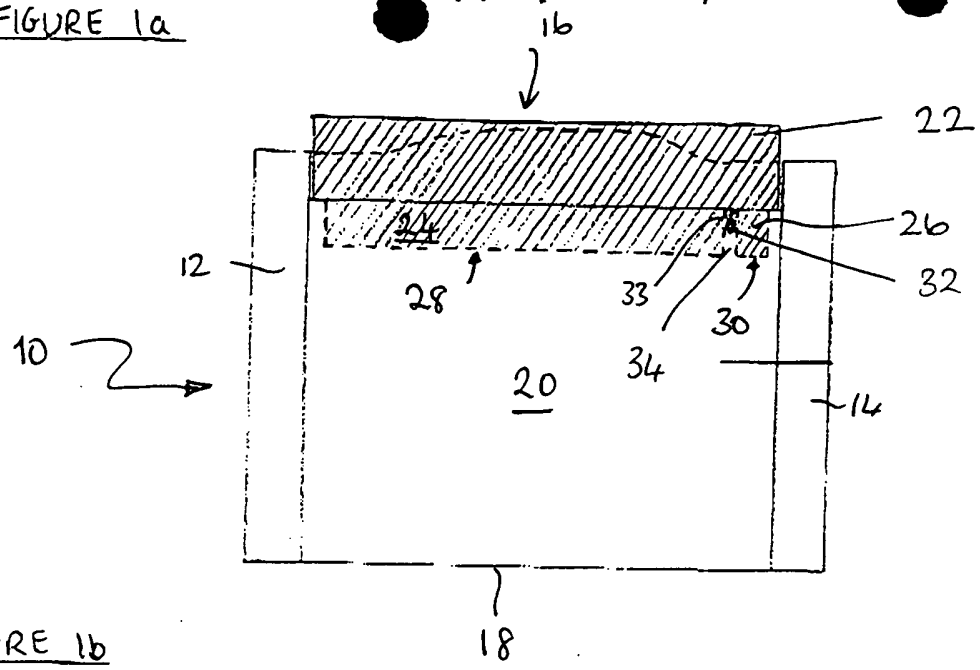

FIGURE 1b

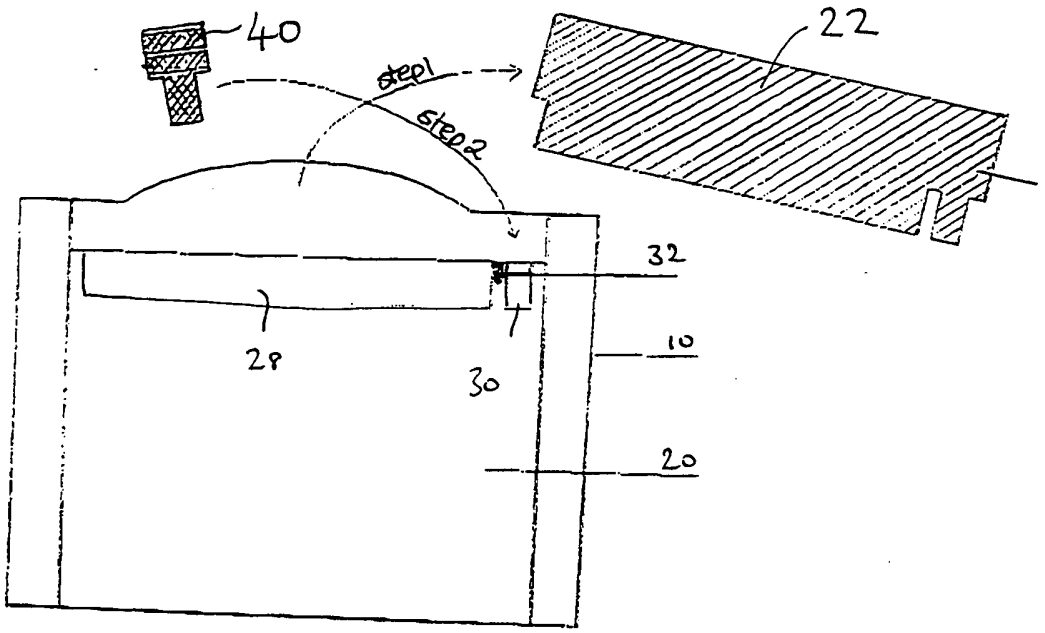

Figure 1c